

Distribution of entomopathogenic nematodes in the Swiss Alps

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Distribution of entomopathogenic nematodes in the Swiss Alps. - A total of 473 soil samples taken from alpine regions in Switzerland was analysed for the presence of rhabditid entomopathogenic nematodes. These parasites were found in 27% of the samples at altitudes between 490 and 2530 m asl. The recovered nematode isolates fall into six species: *Steinernema affinis*, *S. feltiae*, *S. intermedia*, *S. kraussei*, *Steinernema* sp. (a species close to *S. intermedia*), and *Heterorhabditis* sp. (North-West European Group). The distribution of these species is discussed in relation to altitude, vegetation, orientation, soil characteristics (i.e. pH, sand content, content of organic matter), and potential host species. *Steinernema kraussei* was the most commonly encountered species, usually occurring in acidic soils rich in organic matter. *Steinernema feltiae*, the second most prevalent species, was rather confined to grassland habitats of the lower Alps and the Swiss Plateau. Similarly, the other species were usually found in grassland, but were too rare to draw conclusions about their ecological requirements.

Key-words: distribution - environmental characteristics - cold-activity - steinernematids - *Steinernema kraussei* - *feltiae* - *Heterorhabditis*.

INTRODUCTION

Nematodes of the genera *Steinernema* (Steinernematidae: Rhabditida) and *Heterorhabditis* (Heterorhabditidae: Rhabditida) are obligate parasites of soil-dwelling insects. These nematodes include in their life cycle free-living infective juveniles, able to locate and infect suitable host species. Several strains of *Steinernema* and *Heterorhabditis* are commercially sold as biological control agents of various insect pests. However, currently available strains can only be used effectively above temperatures of ca 12 °C (e.g. WOUTS 1991). Problems with the poor cold-activity of entomopathogenic nematodes are common to all countries of temperate zones. To enhance biological control of outdoor insect pests at low temperatures, cold-active nematodes are required. Such nematodes are likely to be found in the Swiss Alps, since alpine animals are adapted to long and cold winters.

The faunistic data presented here refer to a field survey realised as part of COST Action 812 ("Selection and evaluation of cold-active lines of insect-parasitic nematodes for outdoor application"; see EHLERS 1994). Although the present survey was designed primarily to isolate cold-active nematodes, the data available allow us to investigate the distribution of entomopathogenic nematodes in the alpine regions of Switzerland. Moreover, examination of the influence of sample characteristics on nematode prevalence provides insight into the ecology of rhabditid entomopathogenic nematodes.

MATERIAL AND METHODS

A total of 472 soil samples was taken in 1991 at different altitudes in the Swiss Alps. Sampling locations were chosen in order to collect nematodes over a wide range of the Swiss Alps and of some adjacent regions. Sampling in the lower and alpine regions was performed between May and July, and between August and September, respectively. One additional sample was obtained in May 1994 from a small survey in the framework of COST 813 (JENNY 1994). Each sample consisted of 10 subsamples (to a depth of 10 - 15 cm), taken at regular intervals along a transect of ca 50 m. Subsamples were pooled, mixed and a 1 kg portion of the pooled sample was retained to check the presence of entomopathogenic nematodes. At sampling locations below the timberline two samples were generally taken (i.e. one sample within the forest, one on the outside), whereas above the timberline only one sample was taken per location. Entomopathogenic nematodes were baited in the laboratory (at 18 °C) with the wax moth (*Galleria mellonella* L.). For each sample, five late instar larvae, placed on the bottom of a 1-litre plastic pot, were carefully covered with damp soil. After 5 days, the insect larvae were recovered, and placed into plastic dishes on moist filter paper. Nematodes emerging from the *G. mellonella* larvae of each sample (i.e. an isolate) were used to establish laboratory cultures. All isolates were identified using morphological criteria (POINAR 1990; MRACEK 1994), and 35 selected isolates also by restriction fragment length patterns (RFLP of steinernematids by A. Reid, GB-St Albans, Herts; RFLP of the heterorhabditid isolate by P. Smits, NL-Wageningen).

For each sampling location, the following quantitative (a) and qualitative (b) characteristics were recorded: a) altitude, pH, content of organic matter (%C), and sand content; b) region (based on main mountain ranges), orientation (two classes: S-W; NW-SE), vegetation types (woodland samples with subclasses: deciduous, mixed, coniferous with spruce, coniferous with larch; grassland samples: pasture or hay, pasture in vineyard, flowery meadows; "others": home gardens, dwarf shrubland, rosette plants). The pH and %C of soil samples was determined electrometrically in distilled water (using a glass electrode) and by a modification of the Walkley Black method, respectively. The sand content of soil samples was estimated by feel. The weather in 1991 presented generally average conditions, but May was relatively cool and the period between August and September relatively warm and dry (SCHWEIZERISCHE METEOROLOGISCHE ANSTALT 1994).

Ordination and classification of positive samples (entomopathogenic nematodes present) were performed according to similarity in vegetation (two classes: woodland as opposed to grassland and "others") and environmental characteristics (i.e. pH, altitude, sand content, %C, and orientation) using correspondence analysis and complete linkage clustering on square root transformed data (program MULVA-4; see WILDI & ORLOCI 1990), respectively. The Mann-Whitney U-test or the Kruskal-Wallis-test were used to compare rank sums of continuous variables achieved in nominal classes (samples or species). χ^2 contingency table analyses were used to test whether entomopathogenic nematodes occur more frequently in certain classes of samples (i.e. vegetation, orientation, pH, altitude, %C, sand content) than in others. For rare species, the vegetational specificity was analysed with respect to two vegetation types only (i.e. woodland as opposed to grassland and "others"). All comparisons were corrected for ties and used a 0.05 level of significance, unless otherwise indicated. For multiple analyses on the same data set, the Bonferroni correction was applied for critical probabilities (SACHS 1992).

RESULTS

The following species of entomopathogenic nematodes were identified (fig. 1): *Steinernema affinis* (Bovien, 1937), *S. feltiae* (Filipjev, 1934), *S. intermedia* (Poinar, 1985), *S. kraussei* (Steiner, 1923; sensu MRACEK 1994), *Steinernema* sp., and *Heterorhabditis* sp. (North-West European Group, sensu SMITS *et al.* 1991). The latter two species were found only once. *Steinernema kraussei* was clearly the most frequent species (52% or 67 isolates). The isolates denoted as *Steinernema* spp. could not be identified to species, mainly due to contamination and subsequent loss of initial cultures.

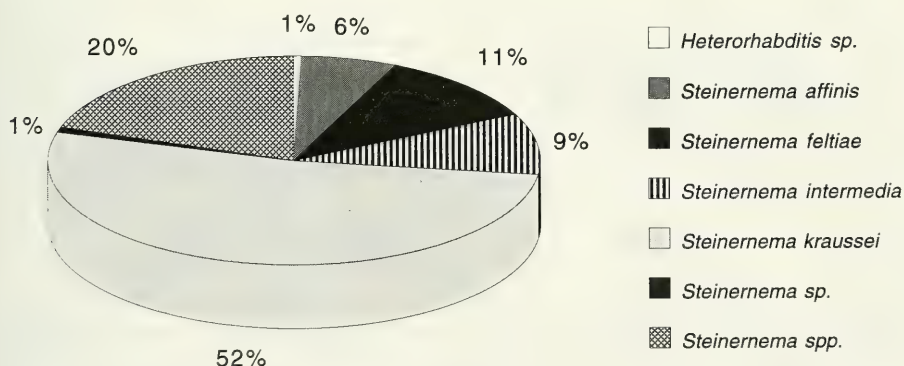


FIG. 1

Species representation of rhabditid entomopathogenic nematodes (n =128) in the Alps and lower Alps of Switzerland. *Steinernema* spp. denote unidentified isolates.

The overall percentage of soil samples yielding entomopathogenic nematodes was 27% (128 isolates in 473 samples). However, nematode frequencies differed considerably among regions, varying between 0.5 and 53% (tab. 1). The variation in prevalence most probably reflects regional differences in sample characteristics, because altitude, pH, %C, and sand content were unevenly distributed over the regions (Kruskal-Wallis-test, $n = 444$, $p < 0.001$). For example, there were no low-land samples (< 1000 m) in the regions R6, R8, and R9 (tab. 1).

TAB. 1

Recovery rates of rhabditid entomopathogenic nematodes in various geographical regions (R1 to R9) of the Swiss Alps related to altitude (asl) of the sampling locations. Regions were defined based on main mountain ranges (see fig. 2).

Regions	Altitude		No of isolates	No of samples	Recovery rate [%]
	Median [m]	Range [m]			
R1: Northern Jura Mountains	805	630-895	2	18	11
R2: Eastern lower Alps	1115	805-1370	4	52	8
R3: Central to eastern lower Alps	850	470-1180	3	60	0.5
R4: Central lower Alps	1130	785-1800	8	78	10
R5: Central to eastern Alps	1270	490-2050	17	52	33
R6: Eastern Alps	1810	1095-2330	39	73	53
R7: Central to southern Alps	1770	720-2320	22	56	39
R8: Central to western Alps	2215	1367-3090	19	45	42
R9: Western Alps	1830	1142-2460	14	39	36
R1 - R9: All regions	1320	470-3090	128	473	27

The regions R1-R4 were invariably characterised by recovery rates clearly below the overall average of 27% (tab. 1). Conversely, the high proportion of positive samples in the five alpine regions (i.e. R5-R9) suggests that entomopathogenic nematodes are very common in the mountain range. The geographical distribution of the 103 isolates identified to the species level is illustrated in fig. 2. The predominant *S. kraussei* is restricted to the alpine regions, whereas *S. affinis*, *S. feltiae*, and *S. intermedia* are more widely distributed. The two species *Steinernema* sp. and *Heterorhabditis* sp. were too rare to interpret their geographical distribution.

The majority of the isolates was recovered in soil samples taken at altitudes between 1500 and 2100 m (fig. 3A). The most elevated recovery site (i.e. with *S. kraussei*) was located at 2530 m. Visual examination of fig. 3A suggests that *S. kraussei* is the most important species in the alpine environment (mean altitude: 1800 m, 1045-2530 m; median and range). The isolates of *S. kraussei* were unevenly distributed with respect to altitude ($\chi^2 = 98$, $DF = 7$, $p < 0.0001$). This was largely due to their high prevalence in altitudinal classes between 1650 and 2550 m, and an absence at altitudes below 1000 m. *Steinernema kraussei* occurred at more elevated sites than both *S. feltiae* (U-test, $p < 0.001$), which predominated in the lower Alps



FIG. 2

Distribution of rhabditid entomopathogenic nematodes ($n = 103$) isolated in the Swiss Alps, in the lower Alps, and in the Jura Mountains. R1 to R9 refer to the regions explained in tab. 1.

Note that isolates from nearby sampling locations are hardly distinguishable.

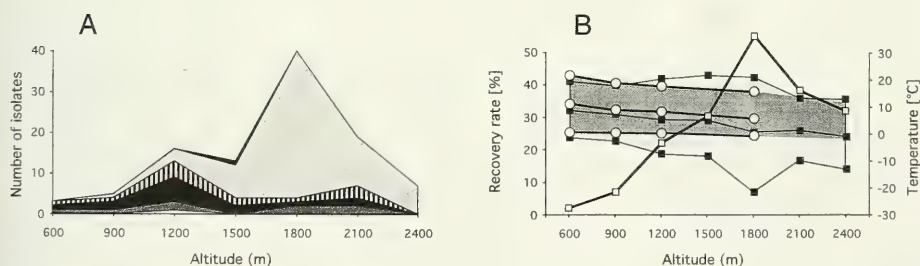


FIG. 3

A) Altitudinal distribution of alpine entomopathogenic nematodes identified at the species level ($n = 103$). For graphical patterns see fig. 1. B) Altitudinal variation of the recovery rate of total entomopathogenic nematodes ($n = 128$) and the zones of variation in air (light grey) and soil temperature (dark grey). Temperature was measured in 1991 at seven climatological stations in Switzerland (SCHWEIZERISCHE METEOROLOGISCHE ANSTALT, 1994). ■: Air temperature (top: mean of the warmest month; middle: annual mean; bottom: mean of the coldest month); ○: Soil temperature at a depth of 5 cm (top, middle, and bottom: see above), values for locations above 1800 m are extrapolated (dashed lines); □: recovery rate of entomopathogenic nematodes related to altitudinal classes.

(1255 m, 580-2170 m), and *S. intermedia* (U-test). No difference was observed in the altitudinal distribution between the other species. *Steinernema intermedia* (1440 m, 605-2205 m) and *S. affinis* (1552 m, 490-2080 m) appear to be uniformly distributed within the zone between ca 600 and 2200 m. *Heterorhabditis* sp. and *Steinernema* sp. were recovered from sites at 1130 and 1530 m, respectively.

The variation in total entomopathogenic nematodes (unidentified isolates included) and temperature changes along the altitudinal gradient are shown in fig. 3B. Values of soil temperature in the coldest months indicate that soil-dwelling animals are well protected from temperatures below the freezing point, irrespective of altitude and associated air temperatures. In the warm season, however, soil temperature decreases at a rate of 1.5 °C per altitudinal class. Therefore, soil-dwelling animals are subjected to similar winter temperatures, whereas during the other seasons less elevated habitats are characterised by considerably higher temperatures than alpine areas. Divergent altitudinal profiles of recovery rate and temperature suggest that the presence of entomopathogenic nematodes depends on factors other than temperature.

The environmental altitudinal gradient represents a complex gradient of temperature (fig. 3B) and other factors related to elevation (e.g. humidity, soil formation). In the present study, for example, altitude was significantly correlated with pH ($r_s = -0.51$, $p < 0.001$), %C ($r_s = 0.21$, $p < 0.001$), sand content ($r_s = 0.44$, $p < 0.001$), and vegetation (Kruskal-Wallis test, $p < 0.001$). Since the nematodes are expected to respond simultaneously to various of these factors, the distribution of the nematodes was analysed using multivariate methods.

Fig. 4 shows the environmental similarity of the positive samples ($n = 125$) based on correspondence analysis and complete linkage clustering. Characteristics of most samples change along a continuum with respect to both axes, and only a few samples are divergent. The main gradient along the first axis represents the transition from sandy soils ("s", to the left) to soils rich in organic matter ("C", to the right). The second axis, accounting mainly for the distinction between *S. kraussei* and the other species, represents a shift from low land samples with relatively high pH levels ("p", top) towards acidic alpine samples ("a", bottom). Vegetation differentiates samples along both axes. Grassland samples ("g", as opposed to woodland samples) are associated with comparatively high pH values, a high sand content, and occur usually at the less elevated sites. The orientation of the sampling locations ("N") is only slightly associated with other sample characteristics. The proximity of "N" to "a", however, indicates that at elevated sampling locations the NW-SE oriented slopes have a slightly higher nematode prevalence than south-facing slopes.

The comparison of species occurrence with sample characteristics within G1 to G5 (tab. 2) provides information about the relative position of each species in the niche defined by the six variables shown in fig. 4. Group 1 (G1) is heterogeneous with respect to entomopathogenic nematodes as it includes all species (except *Steinernema* sp.). The dominating species is *S. kraussei*, representing 67% of the identified isolates. Samples of G1 are characterised by a high organic matter content, and a low sand content. Furthermore, this group includes the majority (i.e. 56%) of all the positive samples taken in woodland soils. In G2, the predominant species is *S. feltiae*,

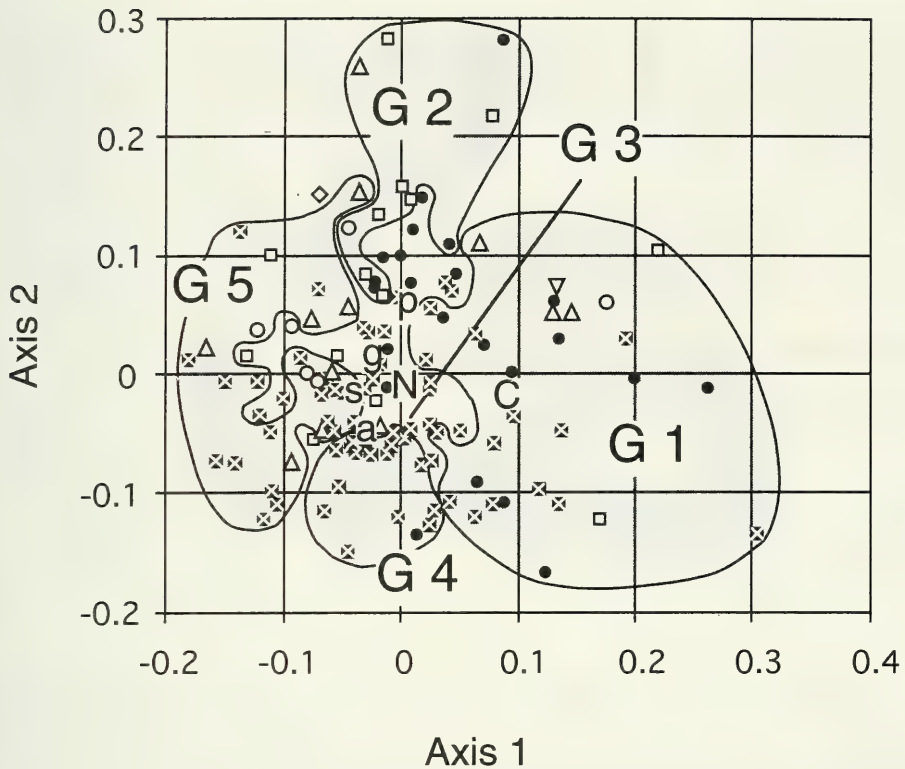


FIG. 4

Ordination and classification of positive samples ($n = 128$) by correspondence analysis and complete linkage clustering, respectively. Samples are characterised by vegetation ("g": grassland and "o": others; as opposed to woodland) and environmental characteristics ("a": altitude; "C": organic matter content; "p": pH; "s": sand content; "N": orientation NW-SE; as opposed to S-W). The eigenvalues of the first two axes are 0.40 and 0.29, respectively. *Steinernema* spp: ●; for other species see fig. 2. G1 to G5: group identification. For clarity, the true boundary of G3 was omitted. The *S. feltiae* isolate located between "s" and "N" is a member of G4.

accompanied by one isolate of *S. intermedia* and *S. affinis*, each. All samples of G2 were taken from grassland habitats at low altitudes and were characterised by relatively high pH values. *Steinernema feltiae* has its main representation in G2 and is thus considered the most adapted species to grasslands in the lower Alps. Note that grassland samples had generally higher pH values (U-test), a higher sand content (U-test, $p < 0.001$), and a lower amount of C% (U-test, $p < 0.001$) than woodland samples.

TAB. 2

Environmental characteristics of the samples belonging to the groups G1 to G5, that were revealed by correspondence analysis and complete linkage clustering (see fig. 4).

Environmental characteristics		G1	G2	G3	G4	G5
pH	(median)	5.5	7.1	6.0	4.8	5.6
	(range)	(4.1 - 7.3)	(5.4 - 7.8)	(4.3 - 8.0)	(4.3 - 5.8)	(4.4 - 7.5)
Altitude	(median)	1565	1100	1800	1917	1765
	(range)	(970 - 1980)	(580 - 1670)	(970 - 2270)	(1610 - 2300)	(890 - 2530)
Sand content	(median)	1	1	1	2	3
	(range)	(0 - 2)	(0 - 4)	(0 - 3)	(1 - 4)	(1 - 5)
Organic content	(median)	13.7	5.8	5.8	7.4	4.5
	(range)	(5.3 - 30.9)	(2.8 - 7.9)	(2.3 - 13.0)	(4.6 - 10.4)	(1.4 - 7.7)
Vegetation (No. of samples) grassland / woodland		13 / 18	13 / 0	25 / 3	14 / 5	28 / 6
Orientation (No. of samples) S - W / NW - SE		4 / 27	3 / 10	18 / 10	1 / 18	3 / 31

The samples of G3 include all the species except *Heterorhabditis* sp., with *S. kraussei* the dominating species (71%). G3 is a transitional group with a majority of grassland samples, characterised by average values for most characteristics except orientation. About 68% of all the samples taken from south-facing slopes (S to W) are included in G3. Note that the overall recovery rate of entomopathogenic nematodes was not the same for the eight radii ($\chi^2 = 24.1$, DF = 7, $p < 0.005$), with a lower prevalence than expected on slopes with a southern orientation. G4 consists of samples yielding almost exclusively *S. kraussei* (94%), accompanied by one *S. feltiae* isolate. Samples of this group were taken at high altitudes and are characterised by low pH values, as well as by a relatively high sand content. The percentage of woodland samples in G4 corresponds to their overall rate in this survey. G5 is dominated by *S. kraussei* (67%) and *S. intermedia* (21%). The latter species has its main representation (i.e. 58%) in this group, and its ecological requirements are presumably similar to those encountered in samples of G5. *Steinernema affinis* and *S. feltiae* are represented in G5 by three and one isolates, respectively. Samples were taken mainly from grassland soils characterised by intermediate pH values, a high sand content, and a low organic matter content.

A more profound insight into the ecological requirements of relatively frequent species can be obtained by comparing positive with negative samples. For *S. kraussei*, this comparison shows that its presence in soil samples is related to orientation, soil characteristics, and vegetation. *Steinernema kraussei* was more prevalent at NW-SE facing slopes than at sampling locations exposed to S-W ($\chi^2 = 10.4$, DF = 1, $p < 0.005$). Moreover, positive samples differed from negative ones by

a higher content of both organic matter (U-test) and sand (U-test, $p < 0.001$), as well as by lower pH values (U-test, $p < 0.001$). The mean pH level of samples yielding *S. kraussei* was 5.4 ± 1.1 (average \pm S.D.). *Steinernema kraussei* occurred in samples characterised by significantly lower pH values than samples with *S. feltiae* (U-test), *S. intermedia* (U-test), and *S. affinis* (U-test, $p = 0.005$). Furthermore, *S. kraussei* seems to have a specificity for the some types of vegetation ($\chi^2 = 26.1$, DF = 7, $p < 0.001$). It was exceedingly frequent in alpine larch stands, but was absent in mixed forests and home gardens, and occurred less than expected in deciduous as well as in spruce forests. In pastures and dwarf shrubland, *S. kraussei* occurred according to sampling effort.

Only one isolate of *S. intermedia* and *S. feltiae*, each, were recovered from forest soils, although woodland samples were relatively frequent ($n = 153$). Both *S. feltiae* and *S. intermedia* seem thus to prefer grassland habitats. Similarly, *S. affinis*, *Heterorhabditis* sp., and *Steinernema* sp. were confined to grassland habitats. However, due to the low prevalence of the species other than *S. kraussei*, their association with the grassland habitat is only significant when they are treated as a group ($\chi^2 = 12.8$, DF = 1, $p = 0.001$). The pH values for *S. feltiae* (6.4 ± 1.2 ; average \pm S.D.), *S. intermedia* (6.2 ± 0.7), *S. affinis* (6.9 ± 0.4), *Heterorhabditis* sp. (6.5), and *Steinernema* sp. (7.8) suggest that these species avoid extreme soil pH conditions.

DISCUSSION

TOTAL ENTOMOPATHOGENIC NEMATODES

Rhabditid entomopathogenic nematodes have been isolated from six continents and appear to be among the most ubiquitous insect pathogens (POINAR 1990). While most steinernematids were isolated from regions characterised by temperate or cool climates, heterorhabditids were mainly recovered from soils in warmer regions (POINAR 1990). In Europe, heterorhabditid and/or steinernematid nematodes occur in all the countries surveyed so far, however, considerable variation exists in the prevalence of entomopathogenic nematodes (BOAG *et al.* 1992). The European surveys demonstrate that these nematodes become scarce as one moves northwards. While they occur in central Europe (i.e. Germany, Switzerland, and Czechoslovakia) with a recovery rate of over 25%, corresponding values are less than 20% in Northern Europe (i.e. Norway, Republic of Ireland, Finland, Northern Ireland, and Scotland; in decreasing order). Only one survey in Sweden with 25% positive samples (BURMAN *et al.* 1986) and another one in Great Britain with 49% (HOMININCK & BRISCOE 1990a) disclaim this tendency.

The observed differences in nematode prevalence may reflect variation in the availability of suitable host species (MRACEK & WEBSTER 1993). However, since most surveys concerning entomopathogenic nematodes are primarily performed to collect new isolates for screening programs, ecological information necessary to explain the distribution of the species is often lacking. Accordingly, sampling of the present survey was designed to isolate cold-active nematodes, and no attempt was made to

identify the natural hosts of the species detected. Another source for the variation in recovery rates between surveys are methodological differences. Since the presence of entomopathogenic nematodes in the soil is difficult to approve when their density is low, the sensitivity of the detection method can substantially influence the estimation of the nematode prevalence. In conclusion, only nematode-positive samples give relevant information on the distribution of the species, whereas negative samples do not provide solid evidence for the absence of the nematodes at a given sampling location. Therefore, analysis of the species' ecological niches using the information of nematode-positive samples (tab. 2, fig. 4) should yield more reliable results than the comparison of positive with negative samples, which served to analyse the environmental preferences of the species.

SPECIES LEVEL

Steinernema feltiae is recorded all over Europe (e.g. MRACEK & JENSER 1988; EHLERS *et al.* 1991; STURHAN pers. comm.). In Northern Europe, *S. feltiae* is usually the most prevalent species (e.g. BOAG *et al.* 1992; GRIFFIN *et al.* 1991; VAINIO *et al.* 1994). In Central Europe, *S. feltiae* represents a subdominant species. For instance in Switzerland, it was the second most prevalent species (fig. 1). The observed predominance of *S. feltiae* in the lower Alps and the Jura Mountains confirms the view that it is a temperate species (HOMININCK & BRISCOE 1990). Complying with its main prevalence in soils with relatively high pH values (G2, tab. 2), *S. feltiae* was found to associate with calcareous soils in Great Britain (HOMININCK & BRISCOE 1990), and in pH neutral soils in Hungary (MRACEK & JENSER 1988). Contradictory findings exist on the vegetational specificity of *S. feltiae*. HOMININCK & BRISCOE (1990) recovered this species from diverse habitats, while in both Germany (STURHAN pers. comm.) and Scotland (BOAG *et al.* 1992) *S. feltiae* was most common in pastures. Results of the present study suggest that *S. feltiae* is associated with grassland habitats (G2, fig. 4). The indicated preference of *S. feltiae* for grassland soils is presumably caused by the distribution of its natural hosts, which include lepidopteran larvae (Noctuidae and Hepialidae) feeding on the roots of tussock grass (POINAR 1990). Fungus gnats and bibionid flies represent other natural hosts of *S. feltiae* (POINAR 1992), which could explain its higher efficiency in parasitising sciarid larvae as compared to the other steinernematid species (STEINER unpublished).

The overall prevalence of *S. intermedia* was similar to that of *S. feltiae* (fig. 1). In Germany, *S. intermedia* is the most commonly encountered species, followed by *S. affinis* and *S. feltiae* (STURHAN pers. comm.). *Steinernema intermedia* was further discovered in Norway (HAUKELAND 1993) and in South Carolina (POINAR 1990). The analysis of its distribution (fig. 4, tab. 2) suggests that *S. intermedia* is a relatively unspecialised species, avoiding extreme pH conditions and exhibiting a slight preference for grassland habitats. In Germany, however, *S. intermedia* was significantly more often found in forests than in other habitats (STURHAN pers. comm.). Therefore, its main distribution in grassland habitats of the alpine region represents not a general vegetational specificity.

Steinernema affinis, *Steinernema* sp. and *Heterorhabditis* sp. were too rare (fig. 1) to allow a detailed interpretation of their distribution in the Swiss Alps. *Steinernema affinis* was recovered in various regions (fig. 2) at altitudes between 500 and 2100 m, and was restricted to grassland samples with pH values near neutrality. In Germany, *S. affinis* was more common than in Switzerland, and occurred most often in pastures and arable soils (STURHAN pers. comm.). This confirms the importance of the grassland habitat for the presence of *S. affinis*. Other records of this species are reported from Norway (HAUKELAND 1993), Denmark (POINAR 1990), the Republic of Ireland (GRIFFIN *et al.* 1991), and Great Britain (REID & HOMININCK 1993). The natural hosts of *S. affinis* include bibionid fly larvae, which this species therefore shares with *S. feltiae* (POINAR 1988).

Steinernema sp. is presumably a new species (close to *S. intermedia*), characterised by the same restriction fragment length patterns (RFLP) as noted for a species recently isolated in Great Britain (A. REID pers. comm.). Its geographical distribution is therefore unknown. *Heterorhabditis* sp. (i.e. North-West European Group) was detected only once in a pasture at 1130 m asl. *Heterorhabditid* species of the North-West European Group occur also in Denmark, Germany, and Poland (SMITS *et al.* 1991). A previously unpublished record of the same species in Switzerland at ca 400 m asl (KLINGLER pers. comm.) suggests that excessive sampling in the Swiss Plateau or in the lower Alps would reveal further isolates of *Heterorhabditis*. The poor representation of *Heterorhabditis* in the Swiss Alps, along with the high frequency of steinernematids, supports the hypothesis that heterorhabditid nematodes are endemic to warmer climates while steinernematids prevail in temperate climates (e.g. GRIFFIN *et al.* 1991; MRACEK & WEBSTER 1993).

Steinernema kraussei was the most commonly encountered species in the Swiss Alps (figs. 1 and 3). Its predominance was unexpected. In Germany, it occurred equally frequent as *S. feltiae*, but was less common than *S. intermedia* and *S. affinis*, while in Great Britain, only 20% of all samples were *S. kraussei* and around 50% *S. feltiae* (A. REID pers. comm.). Since over 25% of the *S. kraussei* isolates in Switzerland originated from sampling locations above 2000 m, this species must endure long periods of low temperatures, and is thus supposed to be a cold active species. In fact, laboratory experiments showed that *S. kraussei* was significantly more active and infective at low temperatures than *S. feltiae* or the heterorhabditid isolate (STEINER 1996).

The alpine distribution of *S. kraussei* indicates the ability of infective juveniles to survive for long periods at low temperatures (BRIAND & WELCH 1963 cited in WOUTS 1991). The permanent snow-cover in winter stabilises soil temperatures near the freezing point. The isolating effect of snow is especially important in regions with extreme minima in air temperatures. In the Engadine (R6 in fig. 2; upper part of the valley of the river Inn), for example, cold air sinking at night to the bottom of the valley leads to very low air temperatures, but soil temperatures are similar to those measured at other climatological stations (fig. 3B; 1800 m). While at 600 m the period with a permanent snow-cover lasts for only 20 days, it increases constantly as one goes upwards, reaching over 250 days above 2400 m. The upper altitudinal limit

of *S. kraussei* could thus be governed by the sum of the positive temperatures in the warmer season, which also affects the duration of the vegetational period and the abundance of associated insect hosts. Evidence is drawn from laboratory observations, showing that 6–8 °C represent the lower limit for the successful propagation of most alpine isolates (STEINER unpublished). This suggests that reproduction is confined to the short summers. The precise role of temperature for the distribution of entomopathogenic nematodes is thus direct via a lower limit for reproduction, and indirect via the vegetation, the availability of associated host species, and soil formation (see below).

The higher prevalence of *S. kraussei* at NW-SE facing slopes as compared to sampling locations exposed to S-W is considered to be a further effect of temperature via soil characteristics. According to the negative relationship between temperature and soil formation, the relatively cool sampling locations oriented to NW-SE were more acidic (U-test, $p < 0.001$) and had a higher content of organic matter (U-test, $p < 0.05$) than sites oriented to S-W.

Surprisingly, though, *S. kraussei* appears to have a lower limit of its natural distribution in Switzerland. Why does *S. kraussei* not colonise areas downslope, all the more as it occurs in Germany (STURHAN pers. comm.) and southern Bohemia (MRACEK 1994) at lower altitudes? Unfortunately, the identity of this species was unclear (e.g. POINAR 1990; MRACEK 1994), and notes on the actual distribution of *S. kraussei* are scarce. Taking into account that laboratory cultures of the alpine *S. kraussei* isolates can be maintained at 25 °C (STEINER unpublished), factors other than high temperature (e.g. presence of natural hosts) must be responsible for the observed lower limit of this species. *Steinernema kraussei* was originally isolated from the web spruce sawfly (*Cephalcia abietis* [L.]) in a German forest stand (MRACEK 1994). The high prevalence of *S. kraussei* in the Swiss Alps, however, must rely on another host species, because *C. abietis* is rare in Switzerland. Occurring exactly in the same altitudinal range as *S. kraussei* in the present survey, *Zeiraphera diniana* (Gn.), a major pest of larch trees, could represent the natural host of this nematode. Since sampling in the present survey was performed only one year after the population collapse of *Z. diniana* in 1990, we can speculate that the larch bud-moth is partly responsible for the high recovery rate of *S. kraussei* in larch stands of the Engadine (R6 in fig. 2). The comparatively low frequency of *S. kraussei* in spruce forest is presumably also related to the availability of suitable host species, all the more soil characteristics of larch stands were similar to those measured in spruce forests.

The analysis of the ecological niche of *S. kraussei* showed that pH and sand content of the soil, the humus, orientation, as well as the vegetation cover influence the distribution of this species. Soil acidity, possibly associated with a high content of organic matter, is considered a key factor for the distribution of *S. kraussei*. Evidence is drawn from a general increase in soil acidity as one moves upwards, paralleled by a habitat shift of the nematode. In the plains, *S. kraussei* seems to prefer woodland soils (e.g. STURHAN pers. comm.), whereas in the Alps, its frequency was equal in grassland and forest habitats. Forest soils differed from grassland at sample sites below 1500 m by a lower pH (U-test, $p < 0.001$), whereas above 1500 m, their acidity

was similar. Conversely, other differences between the two vegetation types remained constant irrespective of altitude. Likewise, woodland samples were characterised by a higher content of organic matter (U-test, $p < 0.001$), and a lower sand content (U-test, $p < 0.05$) than grassland samples.

CONCLUSIONS

The present survey showed that rhabditid entomopathogenic nematodes are represented in the Swiss Alps by six species. *Steinernema kraussei* is clearly the dominant species at high elevations and must be well adapted to alpine climate. It occurs especially frequent in alpine larch stands and occupies an ecological niche well separated from the other nematodes encountered. *Steinernema kraussei* tolerates soils with low pH and rich in organic matter, and is living in both grassland and forest ecosystems, whereas *S. feltiae*, *S. intermedia*, and *S. affinis* are most prevalent in grassland ecosystems at relatively high pH values.

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